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Effect of different cantaloupe (*Cucmis melo* L.) cultivars on whitefly *Bemisia tabaci* (Gen.) digestive enzymes, total protein, total carbohydrates and total lipids

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Abstract
Whitefly *Bemisia tabaci* is considered a serious pest of cucurbit plant in Egypt. It feeds on leaves, flower and fruit of their host plants. It is a suitable model for adapting to the different host plant from intra and inter species. Five biochemical analysis; digestive enzymes (lipase and alpha- amylase), total protein, total carbohydrates and total lipids were carried out to study the effect of three cantaloupe cultivars (Ideal V1, Sundown V2 and Primo V3) on the fourth instar (pupa) of whitefly. The obtained results revealed that whitefly pupa (fourth instar) feed on Primo V3 plant cultivar were found to contain 0.149±0.007, 0.32 ±0.028, and 0.53 ±0.017mg/g fat body weight (F.B.W) in total protein, carbohydrate and lipids respectively, and 0.42 ± .0098, 0.79± 0.017 u/g protein F.B.W of lipase and amylase respectively. These results showed a significant difference than the pupa of whitefly fed on Ideal V1 plant cultivar. whereas, pupa of whitefly fed on Ideal V1 plant cultivar were found to contain (0.106±0.0063, 0.234±0.009, and 0.44±0.011mg/g F.B.W of total protein, carbohydrate and lipids respectively, and 0.38± 0.0057 and 0.50± 0.076 u/g protein F.B.W of lipase and amylase respectively. On the other hand, whitefly pupa feed on Primo V3 has a slight different than insects fed on Sundown V2 plant cultivar (0.129±0.0052, 0.273±.0.007 and 0.49±0.010mg/g F.B.W of total protein, carbohydrate and lipids respectively and 0.42±.008 and 0.75± 0.037 u/g protein F.B.W of lipase and amylase respectively. The current study suggests that *B. tabaci* biotypes utilize different biochemical strategies when encountering various host shifts.

Keywords: Whitefly, digestive enzymes, *Bemisia tabaci*, cantaloupe cultivars, biochemical analysis

1. Introduction
Cantaloupe, *Cucumis melo* L (Fam. Cucurbitacae) is one of the most important vegetable cash crops and have significant economic values [1-4]. Cantaloupe plants are usually infested with various pests which threaten the yield. whitefly, *Bemisia tabaci* (Genn.), (Hemiptera: Aleyrodoidea) is one of the most important limiting factors for cantaloupe cultivation. It is a phloem-feeding got several common names associated with host plants: i.e. the tobacco, cotton, or sweet potato whitefly [5, 6]. It causes direct damage by feeding and indirect damage by acting as a vector for more than 120 plant viruses, particularly begomoviruses [7, 8]. Effect of the different host plant (inter and intra species) on whitefly is very diverse, complicated and no one can know how *B. tabaci* adapts to such a wide range of host [9-13].

2. Material and Methods
2.1. Samples collection
Cantaloupe plants were cultivated at the experimental farm of El-Azhar University **(**Assiut branch**).** Whiteflies (fourth instar) were collected every week during the study period from the beginning of infestation to plant maturity. 4th instar immature individuals were carefully collected from the cantaloupe leaves and weighed to the nearest 0.1g (100mg). The weighted insects were separated and kept in 1.5 ml Eppendorf tubes. The tubes were stored at -80ºC.

2.2. Samples preparation
The collected immature stages were homogenized in 1ml phosphate buffer saline (PBs) (PH 7). Then the homogenates were centrifuged at 10,000 r.p.m. for 10 min.

Supernatant was pooled and taken in three separated eppendorf tubes (V1, V2, V3). Then stored at -20ºC for later use.

2.3. Total Protein assay

Total protein content was estimated according to modified Lowry –Protein Assay [14], using bovine serum albumin as standard. 10µl of insect extract (sample homogenate) was diluted in 1ml distilled water. 400 µl of the diluted sample was mixed with 2 ml of reagent C in a 5 labelled test tubes and left for 10 min at room temperature, then 200 µl of reagent D (foline reagent) was added to each tube. The tubes were mixed strongly and left for 45 min at room temperature. Absorbance was measured at 750 nm on UV spectrophotometer against the mixture of reagent C and D. Amount of protein was calculated from the standard curve. Each assay was done in three to five replicates. Reagent A: 4gm Na_2CO_3+ 0.043gm sodium tartrate + 0.8gm NaOH; Reagent B: 0.045gm $CuSo_4.5H_2O$ that dissolved in 5 ml distilled water; Reagent C: 1 ml of reagent B was diluted in 49 ml of reagent A; Reagent D: 5 ml distilled water was added to 5 ml 2N- Foline ciocateau phenol and stored in refrigerator.

2.4. Total lipids assay

Total lipids were determined calorimetrically by the method according to [15], using kit purchased from Biodiagnostic Company (Egypt)**.** Two tubes labeled as standard and sample, 0.025 ml of supernatant and 1.0 ml of Sulfuric acid concentrated was added into the sample tube. 0.025 ml of Standard to 1.0 ml of Sulfuric acid conc. was added to the standard tube. All test tubes were mixed well, then the tubes was covered by glass beads, and let stand in boiling water bath for 10 min. then cooled. From sample tube, 0.05 ml was pipetted into dry test tubes and 1.5 ml of color reagent was added. From standard tube 0.05 ml was pipetted into dry test tubes and 1.5 ml of color reagent was added. 0.05 ml of sulfuric acid. Finally, 1.5 ml of color reagent was added as a blank test. All test tubes were mixed well and incubated at room temperature for 30 mins in dark. The absorbance of the sample (Asample) and standard (Astandard) was measured against reagent blank at 545nm. Total Lipids Concentration = $(A_{sample}/A_{standard}) \times 1000$

2.5. Total carbohydrate assay

The carbohydrate content was determined according to [16]. 30 µl of supernatant (sample homogenate) was mixed with 500 µl of HCL (18 ml HCL + 32 ml distilled water), then pipetted 50µl of the mixture into labelled tubes. The tubes was covered by a glass beads and let stand in boiling water bath for 1 hour, after removing the tubes from the water bath, 1 ml of Anthrorn reagent was added. Absorbance was measured at 630nm.

2.6. Enzymatic assay

2.6.1. α-amylase activity assay

α -amylase was determined calorimetrically by the method according to [17], using kits purchased from Biodiagnostic Company (Egypt)**.** 1 volume of iodine solution was diluted in 9 volumes of distilled water. In two test tubes labeled as blank and sample 250µl of R1(amylase substrate) was taken and incubated for 3 min at 37ºCin water bath, then 10µl of sample homogenate was added into a sample tube and 10µl of distilled water was added to the blank tube. After mixing well, the tubes were incubated again at 37ºC for 10 mins, then the tubes were removed from incubator and 250µl of working reagent (iodine solution) was added into tubes and mixed well. Finally, 2 ml of distilled water was added to each tube. Absorbance of sample (A_{sample}) and blank (A_{blank}) was measured against distilled water at 660 nm.

α -amylase (U/l) = $(A_{blank} - A_{sample}/A_{blank}) \times 1480$

2.6.2. Lipase assay

Lipase assay estimated according to [18], using chemicals purchased from lab-care diagnostics **(**India). Three tubes labeled as blank, standard and sample. 1ml of reagent1+ (20µl of sample homogenate was added into the sample tube. 10µl of distilled water was added to blank tube and 10µl of the standard to standard tube). After mixing the mixtures were incubated in a heating bath at 37 ºC for 2-3 min then 200 µl of reagent 2 was added and return to the incubator at 37ºC for 2 mins. The absorbance increased for 2 min was measured and Δ absorbance for sample (ΔA_{sample}) and a calibrator ($\Delta A_{calibrator}$) was determined.

Lipase (IU/L) = $(\Delta_{Asample})/(\Delta A_{calibrator}) \times$ calibrator value

2.7. Statistical analysis

All data obtained for biochemical studies were statistically analyzed by using (Graph pad prism 5) computer program calculations at 5% levels.

3. Results

Data summarized and illustrated in Table (1) and Figs. (1-5) showed the changes in; total protein, total lipids, total carbohydrates and enzymes activities; alpha amylase and lipase of 4th instar of *B.tabaci* resulted from switching on different plant cultivars (Ideal V1, Sundown V2 and Primo V3). These results indicated a significant difference at ($p<0.05$) in the previous 5 analyses. The activities were expressed as mg/g protein F.B. W.

In this study protein level in immature stages of *B. tabaci* collected from cantaloupe cultivars Primo (V3) were found to be higher (0.149 mg/g F.B.W) than the total protein level (0.129 mg/g F.B.W) in immature stages of *B. tabaci* collected from cantaloupe cultivar Sundown (V2). While protein levels (0.106 mg/g F.B.W) in immature stages of *B. tabaci* collected from cantaloupe cultivar Ideal (V1) were found to be lower than those in primo and sundown (Table 1, Figure 1). There was a significant difference in protein levels between cultivars Ideal V1 and Sundown V2 and between V1 and Primo V3 but no significant difference between V2 and V3.

In immature stages of *B. tabaci* collected from cantaloupe cultivar Primo (V3), total carbohydrate levels were higher (0.32 mg/g F.B.W) than the total carbohydrate level (0.273 mg/g F.B.W) in immature stages of *B. tabaci* collected from cantaloupe cultivar Sun down (V2). Additionally, the total carbohydrate level in immature stages of *B.tabaci* collected from cantaloupe cultivars Sundown (V2) was significantly high when compared to the total carbohydrate level (0.234 mg/g F.B.W) in immature stages of *B.tabaci* collected from cantaloupe cultivar Ideal (V1) (Table 1, Figure 2).

The difference of total carbohydrate found to be significant between Ideal V1 and Sundown V2 cultivars and between Ideal and Primo V3. On the other hand, no significant difference between Sundown and Primo cultivars (Table 1, Figure 2).

Lipid concentration (0.44 mg/g F.B.W) in immature stages of *B. tabaci* collected from cantaloupe cultivar Ideal (V1) was

found to be lower than the lipid level in immature stages of *B. tabaci* collected from cantaloupe cultivar Sundown (V2) (0.491 mg/g F.B.W). In contrast, total lipid level (0.543 mg/g F.B.W) in immature stages of *B.tabaci* collected from cantaloupe cultivar Primo (V3) was found to be higher than both (V1 and V2) (Table 1, Figure 3). Total lipid level had a significant difference when *B. tabaci* switched on Ideal V1 to Sundown V2 and Primo V3, but no significant difference between Sundown and Primo.

B.tabaci possessed significant lipase activity in nymphal stages. Lipase level in immature stages of *B.tabaci* collected from cantaloupe cultivar Ideal (V1) were found to be lower (0.38 IU/g protein F.B.W) than lipase level (0.443 IU/g protein F.B.W) in immature stages of *B.tabaci* collected from cantaloupe cultivar Sundown (V2), While lipase levels (0.449 IU/g protein F.B.W) in immature stages of *B.tabaci* collected from cantaloupe cultivar Primo (V3) were found to be nearly equal lipase level in immature stages of *B.tabaci* collected from cantaloupe cultivar Sundown (V2) (Table 1, Figure 4). Lipase activity was not significantly different when *B. tabaci* was switched on sundown to primo but had a significant difference when switched on Ideal to sundown and Ideal to primo.

Demonstration of α- amylase activity in *B. tabaci* nymphal stages possessed significant alpha–amylase activity as determined by the tests using starch and iodine solution. The activity of α- amylase was the lowest (0.50 U/g protein F.B.W) in immature stages of *B.tabaci* collected from cantaloupe cultivars Ideal (V1) and highly increased (0.75 U/g protein F.B.W) in immature stages of *B. tabaci* collected from cantaloupe cultivars Sundown (V2), in contrast the increase (0.79 U/g protein F.B.W) in immature stages of *B. tabaci* collected from cantaloupe cultivars Primo (V3) was slightly when compared to sundown V2 cultivar (Table 1, Figure 5). α- amylase activity was significantly different when *B. tabaci* was switched on V1 to V2 and highly significant difference when switched onV1 toV3, but no significant difference between V2 and V3.

Table 1: Physiological results of fourth instar of *B. tabaci* fed on three different cultivars of cantaloupe. The same Lowercase letters followed by mean indicate no significant difference at P> 0.05.

varieties	Total protein (mg/g) F.B.W Mean ±SE	Total carbohydrate(mg/g) F.B.W Mean ±SE	Total lipids(mg/g)F.B.W Mean ±SE	Lipase(IU/g protein) F.B.W Mean ±SE	α- amylase(U/g protein) F.B.W Mean ±SE
V1(Ideal)	0.106±0.0063[a]	0.234±0.0092[a]	0.44±0.011[a]	0.38± .0057[a]	0.50 ± 0.076[a]
V2(Sundown)	0.129 ±0.0052[b]	0.273±.0.007[b]	0.491±0.010[b]	0.423 ±.0088[b]	0.75 ± 0.037[b]
V3(Primo)	0.149 ±0.007[b]	0.32 ±0.028[b]	0.537±0.017[b]	0.429 ± .0098[b]	0.79 ± 0.017[b]

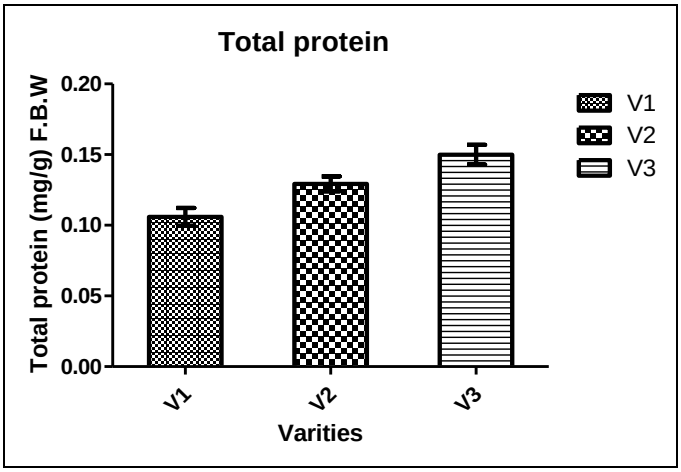

Fig 1: Total protein in *B.tabaci* fed on different plant varieties.

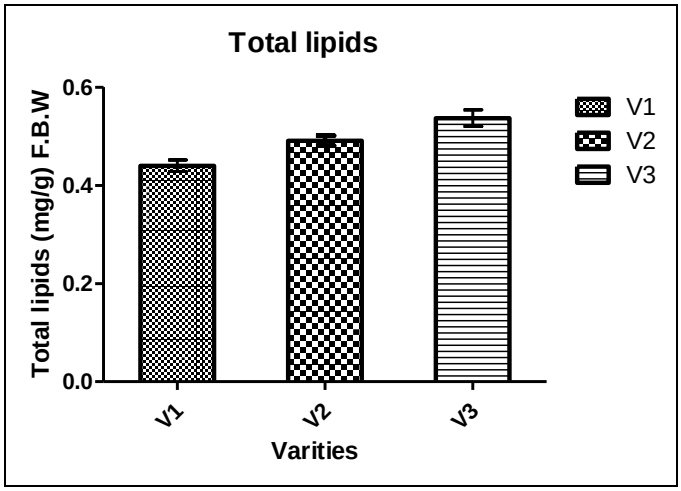

Fig 3: Total lipids in *B. tabaci* fed on different plant varieties.

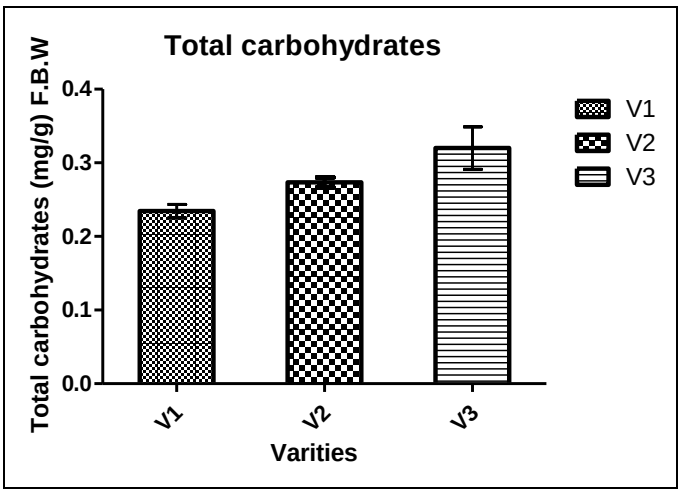

Fig 2: Total carbohydrate in *B. tabaci* fed on different plant varieties.

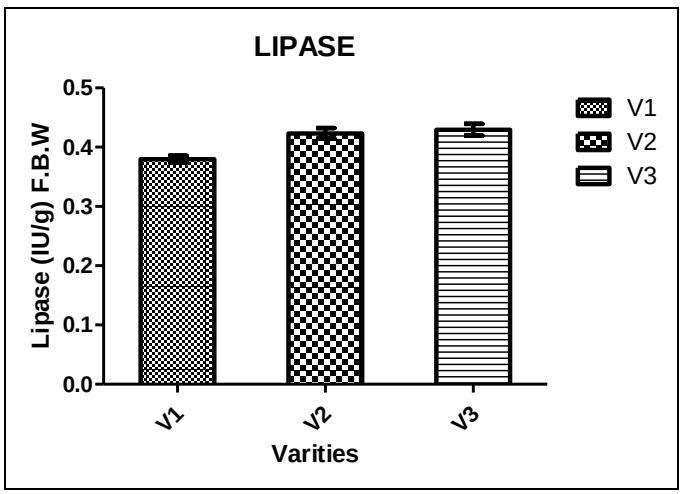

Fig 4: Lipase activity in *B. tabaci* fed on different plant varieties.

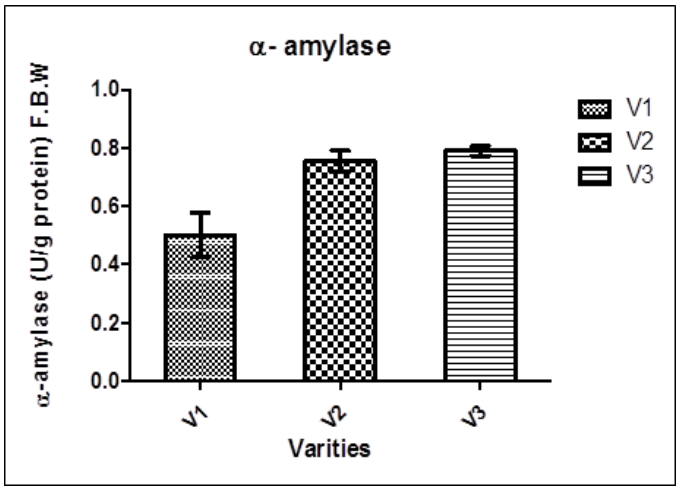

Fig 5: α-amylase activity in *Bemisia tabaci* fed on different plant varieties.

4. Discussion

In insect, the activity of digestive enzymes depends largely on the nature of food resources. The correlation between digestive enzymes and various food resources is an important part of the adaptive nature of polyphagous herbivores [19]. A significant difference in the activity of two digestive enzymes, protease and amylase was noted when the adult of whitefly transferred to Zhongmian 23 from the pre-adapted host zhongmian 41 [20]. These results consistent with [21] who found highly difference in *B. tabaci* after transferring from healthy to Tomato yellow leaf curl China *virus* infected tobacco. This previous results were consistent with the present study which showed a significant difference in the activities of two digestive enzymes, Amylase and lipase when nymphs of *B.tabaci* fed on various cultivars (Ideal, Sundown and Primo) of cantaloupe plants. This results may be due to secondary metabolites of plants. In contrast, Our study showed no significant difference between Sundown V2 and Primo V3. [22, 23] found an increase in both general proteolytic and amylolytic activities when *Helicoverpa armigera* larvae feeding on artificial diet, than when feeding on various host plants. They explain these results due to some secondary chemicals or enzyme inhibitors of these cultivars. On the other hand, [24] reported that plant species had no effect on the proteolytic and amylolytic activities of whitefly. Otherwise, [25] studied the effect of different host on the mirid bug *Apolygus lucorum* an omnivorous species that feed on plants and animals, who stated that the results from food switching experiments proved that amylase activity increased by plant sources, and protease activity increased by animal sources. Thus, the types and activities of digestive enzymes in *A. lucorum* provide the physiological basis of the pest's omnivory.

From the aforementioned results, it is obvious that the switching on plant cultivars caused a significant difference in the total; proteins, lipids and carbohydrates.

In the field, larvae can regulate their intake of protein/carbohydrate by eating a mixed diet of leaves from different plants. This because the protein/carbohydrate content of vegetative tissues can vary within and between plants [26-31] analyzed the responses of *B. tabaci* two types (MEAM1 and the indigenous Asia II 3) after transferring from a suitable host (cotton) to an unsuitable host (tobacco). The results showed that the carbohydrates level were decreased in Asia II 3 while proteins level were increased in MEAM1. In contrast, when transferred *B. argentofilli* between five commercially vegetables; eggplant, tomato, sweet potato, cucumber and garden bean [32] found that the body lengths from 2nd to 4th instars among populations on these 5 host plants were not significantly different. Many previous studies have proved that physiological and biochemical traits of insects can be affected by various plant host shifts [33-36]. Furthermore, [11, 12] stated that physiological and biochemical changes in polyphagous insects induced by phytochemical traits of host plants, such as the composition of the secondary metabolites or allelochemicals, these herbivore responses to hosts change not only among different plant species but also among different cultivars of the same crop species. Consistent with this results our study showed a significant difference in total protein, carbohydrates and lipids when *B. tabaci* nymphs switched on Ideal V1 to Sundown V2 and between Ideal V1 and Primo V3, but no significant difference between Sundown V2 and Primo V3. One possible explanation for no significant differences between Sundown V2 and Primo V3 in the present fifth analysis, this may be due to no bigger variety of changes of allelochemical components and secondary chemicals between V2 and V3 as between Ideal V1 and Sundown V2 and between IdealV1 and Primo V3.

5. Conclusion

In conclusion, The results of this study indicated that there is a considerable variation in total protein, total carbohydrate, total lipids, lipase, and α- amylase activity of *B. tabaci* biotypes when fed on three different cultivars of cantaloupe and forms a good database for further investigations.

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